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VOLUME 5 NUMBER 10

NUTRITION REVIEWS

October 1947

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NUTRITION REVIEWS

VOL. 5 OCTOBER, 1947 No. 10

NUTRITION AND DIETETICS

In 1887 the American Medical Association appointed a Committee on Dietetics and commented editorially (quoted by M. P. Huddleson, *J. Am. Dietet. Assn.* **23,** *573 (1947)*): "Is sufficient attention now being given to it (dietetics) by the American medical student? Or is the average practitioner as well posted as he should be upon these topics"? In four editorials (*J. Am. Med. Assn.* **133,** *1282 (1947);* **134,** *23, 146, 292 (1947)*) this Association has again emphasized the urgency for improved training of the medical student in nutrition.

However, in view of the newer connotations of the words "nutrition" and "dietetics," a discussion of this subject might well have been initiated with a definition of terms. As nutrition is defined, it may be described as the combination of processes by which the living organism receives and utilizes the materials necessary for the maintenance of its functions and for the growth and renewal of its components. It is considered to include the study of the basic problems of chemistry and physiology involved in the needs of the body for food in health and disease.

An understanding of these principles will be essential in medical practice but they will not be enough. In fact, the diagnosis and therapy for nutritional problems will be ineffective unless the medical student is able to translate these principles into foods which are nutritionally appropriate, easily available, and acceptable to the patient. Advice on the selection of foods which are nutritionally appropriate will necessitate an understanding of food composition and the effects of preparation and storage on nutrient content. This is not well understood as indicated in a recent book in which a chart is presented indicating that the "diabetic diet," the "low calory diet," and other therapeutic diets do not meet the recommended allowances of the National Research Council for essential nutrients. That these diets cannot be planned to meet these levels is a gross misconception. Most therapeutic diets can be arranged to meet these recommendations. However, it is implied that therapeutic diets as they are frequently recommended at the present time are below these levels. This may be true. But if so, it is a serious indictment.

In connection with the prescription of therapeutic diets which are appropriate for nutritional efficiency, it will be necessary to recommend changes in food selection and methods of food preparation and storage. To make proper adjustments for the income of the patient, it may be necessary to recommend foods low in cost. To make recommendations appropriate for various occupational, provincial, religious, and emotional needs will involve further adaptability of the dietary prescriptions. This is the field of "dietetics." It is sometimes defined as the combined science and art of feeding individuals or groups under different economic and health conditions according to the principles of nutrition and management.

In diagnosis and in treatment of nutritional problems, dietetics will also play a vital part. In diagnosis, the medical student will, of course, learn to recognize the conditions of the eyes, the tongue, the gums, the skin, and the bony contour as well as the reflexes and nervous stability in nutritional deficiencies. However, since many of the changes are not specific for nutritional deficiencies, confirmatory evidence will be needed from laboratory findings and the diet history. Until simple laboratory tests are available for general use, the chief confirmatory evidence will be gained from the diet history. However, an appre-

ciation is needed of the high degree of skill and understanding required to obtain a reliable diet history. It is entirely insufficient to have a qualitative indication of the food intake such as might be gained from a recital of the menus for the day. Obviously, the diet history will have reliability only if there is a quantitative as well as a qualitative evaluation.

It is in this respect that the dietitian can make an important contribution to the training of the medical student. Tables of 100 g. portions of foods have little meaning to him. He has no adequate understanding of the relationship of these measures to the amounts of foods actually used by the patient. Lack of familiarity with food combinations and recipes frequently place him at a serious disadvantage, particularly with foreign groups. The irregularities in food intake—as on week-ends and holidays—in addition to seasonal and economic factors, also increase the complexities involved in obtaining a reliable diet history. Such additional sources of foods as gardens and chickens must also be considered. In fact, after a due consideration of all the complexities, the medical student may well come to favor the use of weighed diets for a period of time to establish a better clue to the actual food intake, before making a final diagnosis.

Following diagnosis comes specific therapy. The effectiveness of food therapy will depend upon a reliable translation of nutritional principles into foods that are therapeutically appropriate, nutritionally adequate, available, and acceptable to the patient. The acceptability of foods to the patient is now being recognized as the largest single factor in effecting a change in food habits. This will mean that the rigidity of the printed "diet list" must be replaced with elasticity in food recommendations. This in turn necessitates a practical knowledge of food composition, interchangeability of foods, and food combinations.

How can the medical student be given an appreciation and knowledge of the various aspects of food therapy which must be utilized in changing food habits? It is pointed out in the editorials in the *Journal of the American Medical Association* (*loc. cit.*) in the teaching of nutrition, that it will probably be unnecessary to add another member to the staff or an entirely new course to the curriculum. This is also true of dietetics. In many medical schools where clinic facilities are utilized for the teaching of medical students, the nutrition or food clinic has been integrated into such a program. The clinic dietitian is in an ideal situation, not only to teach the basic principles of dietetics, but to vitalize this teaching with demonstration teaching of the patients, and in addition, the medical student himself may be given an opportunity to teach the patient under the supervision of the dietitian.

In the hospital also, the therapeutic dietitian will be in a position to teach the medical student and intern something of the dietary problems involved in the diagnosis and treatment of individual patients.

It has already been shown that the medical student has a need and a motivation for learning something of the practical aspects of therapy in "diet and disease"—in the handling of the low caloric diet, the diabetic diet, the high caloric diet, the bland diet, and so on. And, with a new understanding of food composition and diets as usually consumed by patients, he can be given the tools for a further appreciation and investigation of "nutritional" problems as he finds them in clinical experience.

"Dietetics," then, may be the avenue through which the medical student may obtain a critical appreciation of the content and use of therapeutic diets. In addition, with an understanding of food composition and foods as actually eaten by the patient, he will have a further measure whereby he can ascertain nutritional deficiencies and needs in many other patients.

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PRESENT KNOWLEDGE OF PANTOTHENIC ACID IN NUTRITION

Pantothenic acid was the outcome of several searches for the "bios" effect on yeast, the "antipellagra factor" for chicks, the "antidermatitis (filtrate) factor" for chicks, a liver filtrate factor for rats, and a growth factor for lactic acid bacteria. Particular credit has been given, however, to R. J. Williams and collaborators who isolated a nearly pure sample of the vitamin (as the "bios" factor) from sheep liver, and to R. J. Williams and R. T. Major (*Science* **91,** *246 (1940)*) who determined its structure. Basically, the pantothenic acid molecule is composed of beta-alanine and a hydroxy-dimethyl-butyrolactone.

The vitamin appears to be essential in many species of animals, bacteria, and yeasts, and the deficiency symptoms in animals are so widespread in the various tissues as to indicate a basic function in the metabolism of all cells. In the chick, a prominent sign is dermatitis (A. T. Ringrose, L. C. Norris, and G. F. Heuser, *Poultry Sci.* **10,** *166 (1931)*), but extensive spinal cord lesions, involution of the thymus, and fatty liver have also been described in this species. There is a striking decrease in the hatchability of the eggs when mature laying hens are kept on a pantothenic acid deficient ration, even before the hen shows untoward symptoms (J. C. Bauernfeind and L. C. Norris, *Science* **89,** *416 (1939)*). Furthermore, an amount of the vitamin in the ration considered in excess of requirement further increases the hatchability of the eggs, but at the expense of weight decreases in the brain and heart of the developing chick (see *Nutrition Reviews* **1,** *413 (1943)*). In dogs the symptoms of pantothenic acid deficiency are coma, convulsions, fatty liver, mottled thymus, and gastritis and enteritis (*Ibid.* **2,** *298 (1944)*). In swine there is a striking reduction in growth, loss of hair, ulcerative colitis, and ataxia with sensory neuron damage (*Ibid.* **3,** *269 (1945)*). Deficiencies in other species have been described, and gastrointestinal symptoms seem common to several species.

Pantothenic acid deficiency has not been described in man, but it seems certain that this is due to the ubiquitous occurrence of the vitamin in foods and to the limited studies with purified diets. The widely publicized claims for pantothenic acid in the prevention or treatment of gray hair in man have been denied by controlled studies (*Nutrition Reviews* **1,** *365 (1943);* **2,** *90 (1944)*), but there is no doubt that this vitamin plays a role in achromotrichia in certain animals. This effect may be indirect through an influence on the adrenals and on salt balance. Adrenalectomy completely overcomes the effect of a pantothenic acid deficient diet in so far as hair color is concerned.

The determination of pantothenic acid is usually microbiologic, measuring the growth response of yeast or lactic acid bacteria, although the growth of chicks on the proper ration may be used as a criterion. Microbiologic assay is always preceded by some type of enzymatic liberation of the vitamin from its bound forms since it is generally recognized that the free vitamin is very low in concentration in both plant and animal tissues. Takadiastase and papain have been used extensively for this purpose (V. H. Cheldelin, M. A. Eppright, E. E. Snell, and B. M. Guirard, *Univ. Texas Pub.* **4237,** *15 (1942)*), but recent work on the ineffectiveness of these enzymes in liberating pantothenic acid from "coacetylase" (F. Lipmann and co-workers, *J. Biol. Chem.* **167,** *869 (1947)*) indicates that autolysis during the conventional treatment may be the more important factor.

Subject to possible errors due to the technic of liberation of the vitamin, it has been found that dried yeast, liver, and eggs are particularly good sources of pantothenic acid (100 to 200 micrograms per gram of dry material). Broccoli, lean beef, skim milk, sweet potatoes, and molasses are fair sources

(35 to 100 micrograms), while white potatoes, whole milk, and tomatoes are less efficient sources (20 to 30 micrograms). Among canned foods studied, fish products were the best, and asparagus, peas, and corn were only fair sources. A 57 per cent loss of the pantothenic acid of wheat may be suffered during the manufacture of patent flour (*Nutrition Reviews* **1,** *11* (*1943*)), up to one-third loss during the cooking of meat, and only slight losses in the preparation of vegetables (*Ibid.* **2,** *224* (*1944*)).

Several vitamin analogues of pantothenic acid have been described and reviewed (*Nutrition Reviews* **1,** *234* (*1943*); **3,** *340* (*1945*); **4,** *110* (*1946*)). Among these, pantoyltaurine and phenyl pantothenone are particularly powerful in their bacterial growth inhibitory effects and promising results were obtained in *in vitro* studies with pantothenyl alcohol. In general it appears that alterations in the amino acid portion of the molecule have led to the most successful inhibitors. The promising claim of H. McIlwain and F. Hawking (*Lancet* **I,** *449* (*1943*)) that rats can be protected from 100,000 lethal doses of hemolytic streptococci by pantoyltaurine does not seem to have developed therapeutically.

The relations between pantothenic acid and other nutrilites are not yet clear. Glutamic acid has been observed to enhance the stimulating effect of pantothenic acid or beta-alanine upon a strain of Saccharomyces cerivisiae (N. Nielsen and G. Johansen, *Compt. rend. d. trav. du lab. Carlsberg, serie physiol.* **24,** *61* (*1944*)), while V. Hartelius (*Ibid.* **24,** *185* (*1946*)) showed that glutamic acid acted as an antigrowth substance in the presence of suboptimal amounts of beta-alanine. This effect was overcome by pantothenic acid or additional beta-alanine. A relationship between folic acid, biotin, and pantothenic acid has been exhibited in experiments with rats (*Nutrition Reviews* **2,** *221* (*1944*)). With sulfasuxidine in the diet to repress intestinal vitamin synthesis, the animals developed characteristic pantothenic acid deficiency symptoms, which could not be alleviated by extra pantothenic acid. Addition of biotin and a folic acid concentrate, on the other hand, caused a prompt restoration of growth, recovery from the deficiency symptoms, and a restoration of depleted pantothenic acid liver stores to normal. It was concluded that biotin and folic acid were necessary for proper utilization of pantothenic acid by the rat.

A most significant advance in the subject of pantothenic acid has been the discovery of its coenzyme function in acetylation processes. It had been recognized that pantothenic acid occurred in animal tissue principally in bound form, and also that it is concerned with the oxidation of pyruvate in Proteus morganii and in rat liver (*Nutrition Reviews* **2,** *21* (*1944*)). An activator for choline acetylation was recognized by D. Nachmansohn and M. Berman (*J. Biol. Chem.* **165,** *551* (*1946*)) and by W. Feldberg and T. Mann (*J. Physiol.* **104,** *411* (*1946*)), and a coenzyme for acetylation of sulfanilamide by a liver system was reported by F. Lipmann (*Federation Proceedings* **4,** *97* (*1945*)). This coenzyme was purified by F. Lipmann and N. O. Kaplan (*J. Biol. Chem.* **162,** *743* (*1946*)), and later found to contain as much as 9.3 per cent of pantothenic acid (*Ibid.* **167,** *869* (*1947*)). The coenzyme may have a nucleotide structure, and be concerned with the formation of an "active" acetate capable of acetylating choline and sulfanilamide. It appears that most of the pantothenic acid in animal tissues is in the coenzyme form.

ASCORBIC ACID AND DIHYDROXYPHENYLALANINE METABOLISM

In an earlier article (*Nutrition Reviews* **5,** *173* (*1947*)) the relation between tyrosine metabolism and the supply of ascorbic acid was discussed, with particular reference to the specificity of l-ascorbic acid and that of the derivatives of l-tyrosine which can be metabolized in the same way as tyrosine itself.

R. R. Sealock and T. Ho Lan (*J. Biol. Chem.* **167,** *689* (*1947*)) draw attention to the metabolism of dihydroxyphenylalanine in kidney and liver slices of normal and scorbutic guinea pigs. This problem suggested itself in view of the activity of tyrosine and of dihydroxyphenylalanine as precursors of the pigment melanin. R. R. Sealock, B. Ziegler, and R. L. Driver (*Ibid.* **128,** *lxxxix* (*1939*)) had shown that if either of these amino acids was included in the diet of guinea pigs, the requirement of the animal for ascorbic acid was increased. The question naturally arose, whether 3,4,dihydroxyphenylalanine is metabolized with the aid of ascorbic acid or independently of it.

The experimental method employed was that developed in studies of tyrosine metabolism by Ho Lan and Sealock (*loc. cit.*). Adult guinea pigs were maintained on a diet of Purina chow, devoid of ascorbic acid, with or without the addition of 20 mg. of ascorbic acid daily, and killed for the preparation of tissue slices after either fifteen or twenty-five days of feeding. The Q_{O_2} and Q_{CO_2} (microliters per hour per milligram of dry tissue) values were measured, and the ratio of excess substrate oxygen or CO_2 calculated, in relation to the number of moles of the amino acid present.

Kidney slices were more active than liver slices in the oxidation of dihydroxyphenylalanine, whereas with tyrosine the liver tissue was the more active. The average increase in the oxygen uptake of the tissue over a three hour period was approximately 30 per cent greater in the presence of the amino acid. In a typical instance, kidney slices from a guinea pig fed the basal diet plus ascorbic acid used 7.62 microatoms more oxygen in the presence of 1 mg. of dihydroxyphenylalanine than in its absence, during a three hour test period; the stoichiometric ratio of extra oxygen to dihydroxyphenylalanine was 1.50. This is in sharp contrast to the behavior of kidney slices from a guinea pig kept twenty-five days without ascorbic acid. The oxygen consumption was increased only slightly in the presence of the amino acid, and the ratio of extra oxygen to the amino acid was only 0.149.

After a guinea pig had been rendered deficient, the administration of 20 mg. of ascorbic acid daily for six days restored the ability of its kidney tissue to oxidize dihydroxyphenylalanine. This restoration of oxidizing ability occurs even though ascorbic acid *in vitro* acts as an antioxidant for the amino acid, clearly showing that the presence of the enzyme is essential for the enzymic oxidation of the amino acid. It was also shown that the *in vitro* addition of ascorbic acid to the tissue slices from scorbutic guinea pigs restored their power of oxidizing the amino acid to normal.

Liver tissue differed considerably from kidney tissue; the same degree of oxidation in three hours was not shown, and the extra oxygen consumption of liver in the presence of the amino acid was not significantly different in the scorbutic tissue from that of normal tissue. It may be that an entirely different oxidative system is involved, possibly one which is independent of ascorbic acid. Peculiarly, the relative activities of liver and kidney tissues in the oxidation of dihydroxyphenylalanine are just the reverse of their activities in the oxidation of tyrosine, previously described by Ho Lan and Sealock (*loc. cit.*).

THE DETERMINATION OF BODY WATER *IN VIVO*

Attempts to devise an experimental procedure for determining the body water of the living animal have been made at intervals for some time. Most of them have aimed at finding a readily determined substance which could be introduced into the body and which would then distribute itself uniformly in the total body water. Most of the substances which have been proposed have later been shown to be unsuitable because of their unequal distribution among the different tissues, and have been discarded. It is of interest that the first substance to be used for the *in vivo* measurement of total body water (deuterium oxide) should have been the most successful (G. Hevesy and E. Hofer, *Nature* **134,** *879 (1934)*).

A new method has been devised by N. Pace, L. Kline, H. K. Schachman, and M. Harfenist (*J. Biol. Chem.* **168,** *459 (1947)*), employing "tritiated water," or water containing a small fraction of the isotope containing tritium, a radioactive isotope of hydrogen of mass 3. Preparation of such tritiated water (HTO) has become possible with the availability of beryllium targets which have been bombarded by deuterons for the production of neutrons. Tritium was produced therein by the reaction $Be^9 + D^2 \rightarrow Be^8 + H^3$.

The tritium could be collected as HTO by dissolving the beryllium target in sixth-normal hydrochloric acid in a vacuum system, and then passing the evolved hydrogen and tritium over copper oxide at 550° and collecting the water in a liquid air trap. With ordinary precautions, it was possible to obtain water which was sufficiently sterile and pyrogen-free for intravenous injection into human subjects. Because the volume of the counter tube was constant, values expressed as counts per second were directly proportional to the tritium in each sample examined. The use of tritium was also convenient in that its long half-life (31 years) eliminated the need for any decay correction in the course of the experiments.

The physiologic measurement of body water was made on 2 rabbits and one human subject, and was checked by calculation of the body water on the assumption that the animal and human subjects were normal, containing 73.2 per cent of the mass of the fat-free body. This mass was calculated from the specific gravity of the body and the total body weight, as described by A. R. Behnke, B. G. Feen, and W. C. Welham (*J. Am. Med. Assn.* **118,** *495 (1942)*) and N. Pace and E. N. Rathbun (*J. Biol. Chem.* **158,** *687 (1945)*). This was further checked for the rabbits by direct determination of the total body water by means of desiccation.

The determination of total body water by the new technic was made by injecting a few milliliters of a stock solution of tritiated water after measuring its radioactivity, and then measuring the activity of the tritiated water after dilution by the body water by using a sample of the blood plasma. The results with the rabbits agreed to within a few per cent, whether obtained by the tritiated water dilution, by desiccation measurements, or by calculation from the specific gravity data. The time required for the bulk of the tritiated water to reach equilibrium with the body water is dependent upon the size of the animal in which the measurement is being made. The time for equilibration after injection of the tritiated water into the human subject appeared to be about one hour, the time previously obtained when deuterium was used (F. D. Moore, *Science* **104,** *157 (1946)*). The time for the rabbits was estimated to be not over thirty minutes.

By taking the average for the values for tritiated water dilution at one, two, and three hours in the human subject, a figure of 64.7 per cent was reached for the total body water, in comparison with 65.2 per cent obtained by calculation from the specific

gravity. The authors conclude that the use of the tritiated water technic makes possible the estimation of the body water to within 10 per cent of the true value, an error comparable with that attending the use of deuterium oxide. Much of the error may be attributed to the uncertainty in the estimation of activities of one-half to two times as great as the "background activity," which is unavoidable at present owing to the relatively low activity of the stock solutions used. However, it is to be expected that this error will be reduced with the use of tritiated water of higher specific activity, and with improvements in the technics of measurement.

TRANSAMINASE ACTIVITY IN VITAMIN B_6 DEFICIENCY

Attention has already been given (*Nutrition Reviews* **4,** *232* (*1946*)) to the observations of several groups of investigators whose work led to the conclusion that pyridoxal phosphate is the coenzyme of the enzyme transaminase. Among these studies was that of F. Schlenk and E. E. Snell (*J. Biol. Chem.* **157,** *425* (*1945*)) who showed that the tissues of vitamin B_6 deficient rats were low in transaminase and also that the addition of pyridoxal and pyridoxamine plus adenosine triphosphate reactivated the deficient systems partially in the majority of trials. S. R. Ames, P. S. Sarma, and C. A. Elvehjem (*Ibid.* **167,** *135* (*1947*)) confirmed and extended the observations of Schlenk and Snell (*loc. cit.*).

Observations were made concerning transaminase and succinoxidase in normal and vitamin B_6 deficient rats, with and without the addition of members of the vitamin B_6-complex and their phosphates. Production of vitamin B_6 deficiency was facilitated by use of the ration developed by P. S. Sarma, E. E. Snell, and C. A. Elvehjem (*J. Biol. Chem.* **165,** *55* (*1946*)). Preliminary results showed that rats maintained on the vitamin B_6 deficient diet for six weeks had low levels of transaminase in all tissues examined, which included heart, liver, kidney, brain, and skeletal muscle.

The arbitrary choice of heart muscle and kidney cortex was therefore made as tissues in which to make a more detailed study of the effect of a deficiency of vitamin B_6 upon the transaminase activity of the tissues. Groups of rats were maintained on the vitamin B_6 deficient diet and also on the same diet supplemented with 150 micrograms of pyridoxine hydrochloride per 100 g. of ration. These two groups were kept for six weeks, some of the rats of each group being killed for determination of the transaminase activity of the heart and kidney tissues at intervals of two weeks, by the method of S. R. Ames and C. A. Elvehjem (*J. Biol. Chem.* **166,** *81* (*1946*)). Over the entire period, the rats on the supplemented diet maintained their initial transaminase activities, whereas those of the deficient animals fell to about 40 per cent of the control values. The decrease in activity was most rapid during the first two week period.

One group of rats on the deficient diet was changed to the diet supplemented with pyridoxine, and showed a marked increase in the transaminase activity of the tissues during the last two weeks of the experiment, although the levels did not rise to that of tissues from the rats which had received pyridoxine throughout the six week period. The conclusion was drawn that the rat apparently has only a small reserve of vitamin B_6-complex available for conversion into cotransaminase, since the enzymatic activity of the tissues parallels the growth in showing rapid fluctuations when vitamin B_6 is removed from or returned to the diet.

To find whether the effect was specific for transaminase activity, or whether other en-

zymes were equally affected, the succinoxidase activity of the same tissues was determined both in the deficient and the supplemented rats. The deficient rats showed a slight decrease of succinoxidase activity, to about 80 to 90 per cent of the normal level, thus suggesting a definite specificity of the effect on the transaminases.

The effect of various members of the vitamin B_6 group in reactivating the depressed enzymatic activities of the tissues of the deficient rats was also studied, with results which corroborated the earlier work of Schlenk and Snell (*loc. cit.*). At the lower level of pyridoxal or pyridoxamine addition (*in vitro*), further addition of adenosine triphosphate did not produce any significant increase in transaminase activity. On the other hand, the addition of pyridoxal phosphate or pyridoxamine phosphate caused considerable increases in the transaminase activity.

The investigators have considered their results in the light of comments that this general technic for determining the role of vitamins as components of enzyme systems may be faulty due to unspecific secondary tissue changes. It is suggested that in the application of this technic two criteria should be applied before a relationship is postulated. These are (1) the activity of the enzyme must show a marked decrease in the deficient animal, and on feeding the deficient animal the particular factor, the enzymatic activity must be rapidly regained, and (2) the addition *in vitro* of the vitamin or a derivative thereof must result in a specific reactivation.

URINARY THIAMINE EXCRETION AND THE LEVEL OF THIAMINE NUTRITION

The general question of the urinary excretion of the various vitamins and its relation to the intake of the particular factor has been dealt with in an earlier issue (*Nutrition Reviews* **4,** *86 (1946)*). The work of O. Mickelsen, W. O. Caster, and A. Keys (*J. Biol. Chem.* **168,** *415 (1947)*) has added a wealth of data to previous knowledge of the relation of excretion to intake, although the data are restricted to thiamine alone.

These authors point out that most of the work on this subject has extended only over short periods of time, making it likely that the subjects had not attained equilibrium in their urinary excretion of thiamine. In addition, it is claimed that in many cases the methods for the determination of thiamine in the urine are open to some question. Finally, it is pointed out that even when data were produced which were satisfactory in these respects, and extensive enough to warrant it, such data rarely were treated by appropriate statistical methods which might be expected to reveal all the information implicit in them.

They therefore present their own data, collected over a period of more than three years on the excretion of thiamine and pyramin (the pyrimidine-like moiety of the thiamine molecule which is excreted in the urine). The experiments were made with healthy young men as subjects. In the first experiment, 2, 4, and 4 men were given 1.81, 1.01, and 0.61 mg. of thiamine per day respectively for a period of twenty-four weeks. In the second, two groups of 6 men each received 1 and 2 mg. of thiamine respectively per day for a period of thirty-four weeks after a control period of a month on the lower level of thiamine intake. The intake levels of the two groups after a period of one month on a partially controlled diet were then reversed.

In each of these experiments, all men received the same basal diet, which was served in weighed portions from the diet kitchen. The differences in thiamine intake were adjusted by means of thiamine pills and placebos. The diet itself contained about 0.5 mg. of thiamine daily; the actual analysis of the daily portions revealed that the standard de-

viation of this amount was between ±0.06 and ±0.08 mg. daily. The excretion levels at higher levels of intake, 5, 11, and 16 mg. of thiamine daily were later measured on other subjects receiving less strictly controlled thiamine intakes.

In all of these experiments, except where the intake was over 5 mg. daily, diet samples were collected daily in the form of duplicate meals, which were analyzed by the method of D. J. Hennessy and L. R. Cerecedo (*J. Am. Chem. Soc.* **61,** *179 (1939)*). All the urinary excretion data were obtained on twenty-four hour samples collected in bottles containing 5 ml. of toluene and 5 ml. of glacial acetic acid, by the thiochrome procedure as modified by O. Mickelsen, H. Condiff, and A. Keys (*J. Biol. Chem.* **160,** *361 (1945)*). The pyramin of the urine was determined by a modification of the yeast fermentation method of A. S. Schultz, L. Atkin, and C. N. Frey (*Ind. Eng. Chem. (Anal. Ed.)* **14,** *35 (1942)*).

The statistical analysis of the data obtained revealed that certain of the facts concerning the thiamine excretion are basic and yet have been too often neglected. In the first place, the attainment of excretion equilibrium at a given intake level takes many days; the evidence obtained suggests that on the average only half the change in excretion following a change of daily intake has taken place in ten days. Since the change follows an exponential course, a much longer period (about six weeks) is necessary for its completion. The authors feel that they do not yet have any "proper information as to variations in the rate of attainment of equilibrium." They were however able to show that when equilibrium had been established at a constant thiamine intake, there were large individual differences and considerable day-to-day variations. Consequently they feel that the limitation of the analyses of data to what they call "a casual comparison of group averages" has led to unwarranted optimism with regard to the precision with which the body stores of thiamine can be estimated from excretion data.

Since one "normal" subject may excrete three times as much thiamine as another, these differences are likely to be considerably larger than the mean differences. Presumably in survey work, where the subjects may be expected to be a less rigidly "normal" and homogeneous population than that examined by the authors, the variation might be even greater.

The difference between the excretion of thiamine and of pyramin appears to make the latter more suitable for estimating the thiamine nutrition of the individual. Thus the thiamine excretion levels appear to be related linearly to the thiamine intake, although as emphasized above they are highly characteristic of the individual. All variabilities, between individuals as well as from one day to another for a given individual, increase markedly at higher intake levels. The pyramin excretion values increase exponentially as thiamine intake increases, with the curve reaching a value of 400 micrograms of pyramin excreted at high intake, although at usual levels (1 to 2 mg. thiamine ingested daily) the relationship of pyramin excretion to thiamine intake is close to linear.

With the pyramin data the statistical treatment was found to be simpler, giving an increase in the certainty of the results. An additional advantage was that the individual differences were found to be small, whereas the thiamine excretions exhibited large individual differences. Finally, the pyramin excretion has the advantage that it remains measurable at low intakes of thiamine, whereas the thiamine excretion falls to zero.

Thus this study shows that in nutrition survey and similar work there are distinct advantages in estimating the state of thiamine nutrition by following pyramin excretion rather than that of thiamine excretion. At the same time the more general conclusions drawn are also of considerable value; they concern the necessity for statistical treat-

ment of the data before any general conclusions regarding the relation of excretion to intake can be drawn. In particular, they emphasize the necessity of recognizing the nature of the variabilities involved in the basic data.

PLACENTAL METABOLISM OF ASCORBIC ACID

Because studies of C. T. Javert and H. J. Stander (*Surg. Gynec. Obst.* **76,** *115* (*1943*)) and of W. E. King (*Ibid.* **80,** *139* (*1945*)) implied a possible etiologic relationship between ascorbic acid levels and spontaneous abortions, J. H. Holzaepfel and A. C. Barnes (*Am. J. Obst. Gynec.* **53,** *864* (*1947*)) undertook to investigate the placental metabolism of this vitamin. Gross analyses revealed no constant factor relating the maternal blood levels with placental concentrations of ascorbic acid (A. C. Barnes, *Ibid.* **53,** *645* (*1947*)). Since a relationship between ascorbic acid and spontaneous abortion would presumably depend more on the vitamin concentration and possibly localization in the tissues involved than simply on the levels discovered in the maternal plasma, histochemical analyses were made. In doing these it proved necessary not only to establish the normal in the term placenta but also to review critically the technics available for ascorbic acid localization.

In the publication cited, Holzaepfel and Barnes present a technic for a histochemical analysis for ascorbic acid which depends on the reducing action of ascorbic acid on silver nitrate. The method has been studied by these investigators in detail and the reasons for and probable errors involved in the various steps in the method are evaluated carefully. The significance of the solubility of ascorbic acid in the various reagents used is considered, as well as the degree of tissue penetration attained by the silver nitrate solution and the effect of the presence of other reducing substances in the placenta which might give false reactions. In evaluating these difficulties in the staining technic and establishing a suitable one, over five hundred placentas were examined.

Application of the modified technic to the human placenta revealed that the greatest concentration of ascorbic acid lies at the syncytial layer of the villus. A smaller portion was demonstrated within the stroma of the villus but there was consistently more of the vitamin in the fetal than in the maternal portion of the placenta. This distribution is a reflection of the gradient of physiologic activity in the villus. Most of the metabolic functions occur at the syncytial layer, the area of maternal-fetal interchange, and in term placentas the principal evidences of degeneration and cytolysis lie in the center of the villi.

In comparing these observations with other reported localizations within placental tissue it may be noted that ribonucleoprotein and alkaline phosphatase are both likewise found predominantly in the syncytial layer. Their relationship is an inverse one, however, the ribonucleoprotein being high in early pregnancy and decreasing as the alkaline phosphatase rises (E. W. Dempsey and G. B. Wislocki, *Am. J. Anat.* **76,** *277* (*1945*)). In the studies which Holzaepfel and Barnes made by interrupting pregnancies in rabbits at thirty and sixty days no differences in concentration of ascorbic acid were determined which could be related to the duration of the pregnancy. It is of interest that Dempsey and Wislocki have postulated that the syncytium is the site of the production of placental steroids on the basis of histochemical evidence. Glycogen, on the other hand, is not laid down primarily in the syncytial areas, being found principally where vascularization is poor and oxygen tension low. Iron is distributed in both syncytium and stroma, not showing the sharp gradient which was observed in the case of ascorbic acid.

EFFECTS OF RESTRICTED AND SUPPLEMENTED DIETS IN HUMAN SUBJECTS

During World War II, a number of unusual opportunities for conducting experiments on the effect of variation in nutrients on physical well-being and performance of human subjects arose. Some of these have been reported in this journal (*Nutrition Reviews* **2,** *115, 334 (1944);* **3,** *5, 221 (1945);* **4,** *341 (1946);* **5,** *129 (1947)*).

An extensive study of this nature has been reported by G. H. Berryman and co-workers (*Am. J. Physiol.* **148,** *618 (1947)*). The experimental subjects were 7 young men on detached service, each of whom had volunteered for the study and whose cooperation during it received the highest praise from the authors. During an initial control period of twelve weeks on a normal diet, and at intervals during the subsequent experimental period, levels of physical performance were obtained by means of an electrodynamic brake bicycle ergometer, a treadmill, and the Harvard step test. Tests of psychomotor or psychologic response were also made in a number of different ways, including the Johnson code test, a single dimension pursuit test, etc. A number of analyses of urine, blood, food, and feces were performed at frequent intervals for the various vitamins of the B-group, for tryptophane, lactic acid, pyruvic acid, creatinine, ascorbic acid, etc. Clinical evaluations of physical condition were made periodically by physical examination, measurements of electrocardiograms, and basal metabolic rates.

Following the twelve week control period, the men received daily for thirty-five weeks a diet containing approximately 3000 calories and restricted in levels of B-complex vitamins and protein to levels considerably below the recommended levels of these nutrients (thiamine 0.5 mg., riboflavin 0.3 mg., niacin 5.8 mg., synthetic folic acid 23 micrograms, pyridoxine 1.1 mg., pantothenic acid 1.1 mg., biotin 19 micrograms, and protein 45 g., of which 94 per cent was of non-animal origin). After five weeks of the restricted intake, 2 of the subjects were chosen as controls, and received thereafter supplements of nutrients in amounts which made their total intakes equal to or slightly higher than the levels during the control period. Placebos of identical appearance were administered to the remaining experimental subjects. After fifteen weeks of the experimental period, 3 of the remaining subjects were given supplements of thiamine alone, while the other 2 subjects were given similar supplements two weeks later. At intervals thereafter, additive supplementation with protein, riboflavin, niacin, and the lesser known B-complex vitamins was made at times which varied from subject to subject. After twenty weeks of this supplementation schedule (thirty-five weeks of the experimental period) a "luxurious" diet was fed ad libitum for three weeks, making the total elapsed time of the experiment, including the control period, fifty-one weeks.

At the end of fifteen weeks on the experimental diet, definite decreases in the amount of work performed on a bicycle ergometer and elevation of resting and postexercise pulse rates had occurred in all experimental subjects. Some were also unable to complete the step and treadmill tests which they had been able to perform with relative ease during the control period. The control subjects showed either moderate improvement or no change from the normal diet period on each of these tests. Body weight of experimental subjects declined only slightly. Symptoms reported by the subjects were lassitude, insomnia, sore mouth, anorexia, nausea, pyrosis, vague paresthesias, and severe cramping pains of the extremities, none of which was reported by the control subjects. Personality appraisal scores indicated a change toward hysteria, depression, and hypochondriasis in the experimental subjects but not in the controls.

Analytical results showed that urinary excretion of the vitamins fell off rapidly following change to the deficient diet, but the levels of vitamins in the feces remained essen-

tially unchanged. Positive nitrogen balance was maintained throughout the experiment. Despite its low level in the experimental diet the excretion of free l-tryptophane did not decrease from that found in the normal diet period.

When the experimental subjects received supplementation with crystalline nutrients their urinary vitamin excretion levels soon returned to or exceeded normal. In contrast, improvement in physical performance tests occurred only gradually. No one nutrient produced striking recoveries in any of the subjects. Improvement in physical performance and in personality was more rapid in subjects receiving supplementation with the complete vitamin mixture than in those receiving stepwise addition of nutrients. The pains of the lower extremities disappeared slowly as vitamin B-complex supplementation was received, but by the end of twenty weeks of supplementation these symptoms had subsided almost completely. All other clinical signs and symptoms which had not regressed earlier also disappeared, but it was not possible to associate their disappearance with supplementation with any single nutrient. Further improvement in physical performance occurred during the terminal three week period of the experiment, when the subjects ingested a rich diet.

The authors emphasize the variability of individual response of the various subjects, and warn against application of the concept of numerical average in experiments of this type. They also emphasize the marked slowness in recovery of ability to perform the amount of work within the original capability of the individual, even when the complete supplement was given. No portion of the recovery curve was steep until the luxurious diet was fed, and no "magical" effect of any vitamin or combination of vitamins, or of protein, was observed.

No definite answer to the role of the lesser known B-vitamins (i.e. those other than thiamine, riboflavin, and niacin) in human nutrition was gained from the study, but it was apparent that improvement of physical performance was more rapid and permanent when the lesser known vitamins were provided as a group together with thiamine, riboflavin, niacin, and protein.

VITAMIN B-COMPLEX AND WORK OUTPUT OF PERFUSED MUSCLE

The important role played by a number of the vitamins of the B-complex group in the enzyme systems which are involved in the energy transformations in the living organism makes it seem possible that a direct decrease in the work output of muscle might result from dietary deficiencies.

There is no doubt that relatively severe or prolonged dietary inadequacies lead to deterioration of physical fitness and efficiency. However, questions arise as to the degree of deficiency, the factors involved, and the length of the period of the deficiency which will lead to a decreased work output. Furthermore, there is a question as to whether the effect is related directly to the biochemical defects of muscle physiology or to other indirect influences. The scientific literature is sparse and controversial on most of these questions.

A number of investigators report an increased fatigability, deterioration of physical fitness, and decreased work output within a few days or weeks after certain of the B-complex factors are missing from the diet, or present in only small quantities (*National Research Council Bull.* **109** (*1945*)). Others have failed to obtain such results. Part of this apparent disagreement may lie in the difficulty of measuring fatigue, work output, or physical fitness. Some of it undoubtedly lies in the difficulty of eliminating psychologic and training factors from the measurements when dealing with human

subjects. Finally, even if changes are detected with certainty they may be referable to changes in the body as a whole. While such observations are useful, they usually do not supply the information which is necessary for understanding the underlying physiology. It is evident that new technics would be helpful in the search for satisfactory answers to the problems raised above. Shock and Sebrell, in a series of experiments reported during the last three years (N. W. Shock and W. H. Sebrell, *Am. J. Physiol.* **142,** *265, 274 (1944);* **146,** *399 (1946); Proc. Soc. Exp. Biol. Med.* **59,** *212 (1945);* **63,** *290 (1946)*) have made a probe in this direction. They have studied the effects of the various members of the B-complex on the work output of standardized frog muscle preparations and have found some interesting if not as yet explainable results.

The work output of isolated gastrocnemius muscles of frogs perfused with glucose-Ringer's solution has been compared with the work output of the same muscles of the opposite legs perfused with glucose-Ringer's solution containing variable amounts of the various vitamins of the B-complex. Thiamine, cocarboxylase, pantothenic acid, pyridoxine, and riboflavin when present in small concentrations (0.01 to 0.0001 mM) in the perfusion fluid lead to an increased work output of the muscles of approximately 20 per cent over that of the corresponding controls. The presence of the vitamins enhances the height of the individual contractions and delays the appearance of fatigue (decrease in muscle contraction after eight to ten minutes). Minimal effective concentrations of 0.0001 mM were found for riboflavin, pyridoxine, and cocarboxylase, while the corresponding figure for thiamine was 0.005 mM and for pantothenic acid 0.001. As the concentrations were increased above these values a zone of optimal effect was observed, beyond which further increases in concentration led to a decreased effect and finally to no effect at all. The optimal effective mM concentrations found were 0.0001 for pyridoxine, 0.0005 for riboflavin, 0.001 for thiamine, and 0.01 for pantothenic acid. Concentrations beyond 0.005 for pyridoxine, 0.01 for riboflavin, and 0.75 mM for thiamine and pantothenic acid gave no enhancement at all.

The available data are inadequate for comparison of these concentrations with those of the fluid which normally bathes the muscle cells. However, rough estimates would place the experimental figures at about ten to a thousand times the physiologic levels.

In view of the care with which the measurements were made and the data obtained, there seems little doubt of the reality of the effects observed. All points were established by measurements on at least ten pairs of muscles in which the opposite muscle in the same animal always served as control. The work output of the opposite muscles of individual animals was determined and the standard error of the mean differences was calculated and found to be significant. Possibilities that slight changes in pH or ion concentration of the perfusion fluid or other such factors might be responsible for the effects were explored with negative results. The concentrations of the substances tested were so low that it seems unlikely that they could contribute significant energy as substrates.

It is of interest that all of the substances tested gave about the same response at optimal concentration (20 per cent increased work output) and that this figure was not exceeded when a mixture of them was included in the perfusion fluid. This would seem to indicate that some other factor involved in muscle metabolism was limiting the effects of the added vitamins to the 20 per cent level. Ample glucose and oxygen were present in the perfusion fluid. Tests with 20 units of insulin per liter of fluid gave no support to the possibility that insulin might be a limiting factor.

It is also puzzling that so many substances of different chemical structure which are usually characterized by the specificity of

their physiologic reactions should give the same effect. Since these are short-time measurements (fifteen minutes) and since it is likely that all of the substances concerned are involved in some way in muscle metabolism, it is conceivable that any one of them might sufficiently stimulate the particular metabolic step in which it normally functions to provide a temporary increase in metabolites available for supporting contraction. This, however, is a mere speculation on the part of the reviewer.

The fact seems to be that here is a real and interesting unexplained observation which although a puzzle at the moment, may provide the student of muscle biochemistry in due time with an important clue and technic.

THE MICRODETERMINATION OF ASCORBIC ACID IN WHITE BLOOD CELLS AND PLATELETS

Interest has been shown in the ascorbic acid content of the white blood cells ever since D. J. Stephens and E. E. Hawley (*J. Biol. Chem.* **115,** *653 (1936)*) showed that these living cells normally have a relatively high concentration of this substance. In their work, the analysis was made upon the buffy layer of cells derived from a 50 ml. blood specimen. This layer contains both white cells and platelets, which were shown by A. M. Butler and M. Cushman (*J. Clin. Invest.* **19,** *459 (1940)*) to have almost the same concentrations of ascorbic acid. The macro procedure for the analysis has subsequently been refined until R. Lubschez (*Ibid.* **24,** *573 (1945)*) succeeded in using as little as 4 or 5 ml. of blood.

Meanwhile the significance of the white cell ascorbic acid concentration gradually assumed increasing importance as an index of ascorbic acid nutrition, an importance much increased by the comprehensive study of O. H. Lowry, O. A. Bessey, M. J. Brock, and J. A. Lopez (*Nutrition Reviews* **5,** *185 (1947)*). This study was made with the aid of a new micromethod which had not then been described, but which has since been described by O. A. Bessey, O. H. Lowry, and M. J. Brock (*J. Biol. Chem.* **168,** *197 (1947)*).

The major difficulties encountered in developing the method involved the preparation and measurement of the sample of white blood cells. The method of isolation of the white blood cells (this term is used throughout to signify a mixture of white blood cells and platelets) was a modification of that employed by A. T. Gorham, J. C. Abels, A. L. Robins, and C. P. Rhoads (*J. Clin. Invest.* **21,** *161 (1942)*), who allowed undiluted, oxalated blood to stand for several hours until the red cells were partially settled, and then centrifuged the supernatant layer. In very small quantities it was preferable to dilute the sample with isotonic potassium oxalate to increase the yield of white cells and to permit more complete removal of serum without washing the cells. The red cells were first separated by slow centrifugation rather than by allowing them to settle out upon standing.

The size of sample was determined by an indirect method; it was found by experiment that the concentration of acid insoluble phosphorus was relatively constant in the white blood cells. The amount present, in macro determinations with white cells, was close to an average value of 3.34 mM per 100 g. Although there was some variation, so that correlation between this phosphorus fraction and the wet weight of cells was not perfect, it was thought possible by the authors that the acid insoluble phosphorus might actually represent a more physiologic basis for calculation than weight.

In the new method, therefore, the acid insoluble phosphorus of the cells is determined routinely as well as the ascorbic acid, for the calculation of the weight of the

sample. After the ascorbic acid has been extracted from the cells by means of 5 per cent trichloracetic acid, the residual cells are washed with 0.2 to 0.3 ml. of 5 per cent trichloracetic acid, centrifuged, and the supernatant removed. The cells are then washed with sulfuric acid and perchloric acid, and treated with a small volume of phosphate reagent. Blanks and standards with known amounts of phosphorus are of course prepared at the same time. The manipulation and handling of the small volumes of cells or of solutions are carried out with the aid of the Levy-Lang constriction pipets and microtubes customarily used by these investigators, and the final color measurements are made in the Beckman spectrophotometer with the aid of special adapter cells for handling minute volumes (*Nutrition Reviews* **5,** *71* (*1947*)).

Figures are given for comparison of the new micromethod with the macromethod of Butler and Cushman (*loc. cit.*), to show that there is close agreement between the results, giving a standard deviation of 3.6 mg. per cent or a coefficient of variation of about 13 per cent. The coefficient of variation of individual microanalyses was only 8 per cent.

When it is stated that the new method is sufficiently convenient and rapid that two analysts can perform fifty to seventy-five analyses in two days, it is evident that the method should be of great value in nutrition survey work.

EFFECT OF PROTEIN HYDROLYSATES ON FOOD CONSUMPTION

Following the intravenous administration of protein hydrolysates as a supplementary source of nitrogen, physicians have often observed that patients eat less food. In this way the expected benefit from the intravenous hydrolysate is diminished. C. J. Smyth, A. G. Lasichak, and S. Levey (*J. Clin. Invest.* **26,** *439* (*1947*)) measured the effect of both orally and intravenously administered protein hydrolysates and of a synthetic amino acid mixture on the voluntary food consumption of 8 normal adults and one patient with portal cirrhosis of the liver. One of the hydrolysates was an enzymatic digest of casein and pork pancreas ("Amigen"), the other an acid hydrolysate of casein supplemented with tryptophane ("Parenamine"), while the amino acid mixture contained only the ten "essential" amino acids and glycine. Voluntary consumption from weighed diets was converted into food values using standard tables.

After a suitable control period of the diet alone, supplemental daily oral or intravenous feedings of 100 g. (N x 6.25) of one of the hydrolysates were given for from three to seven days. The experiment was then repeated two or three times, usually with a different preparation, and each time with an intervening control period. In a few instances intravenous saline or 10 per cent dextrose was given instead of one of the protein preparations. The rate of intravenous administration of the two hydrolysates varied from 13 to 40 g. of protein per hour, that of the amino acid mixture from 22 to 40 g.

The oral administration of the enzymatic hydrolysate appeared to have little effect on the food consumption, while both protein hydrolysates administered intravenously decreased the amount of food eaten. The effect was minimal with the enzymatic hydrolysate but decreases of up to 63 per cent of the control food consumption occurred following the acid hydrolysate. In the three studies using the synthetic amino acid mixture, no change, an increase of 12 per cent, and a decrease of 15 per cent, respectively, were observed. No selective reduction of protein, carbohydrate, or fat was observed. Saline and 10 per cent dex-

trose intravenously did not appear to alter the food intake significantly.

Nausea and vomiting occurred following administration of the acid hydrolysate in the six instances where the observation was made. Unfortunately, complete information in this respect was obtained in only two instances following the enzymatic hydrolysate: nausea in one, and no symptoms in the other. The amino acid mixture produced no nausea or vomiting. Administration of the acid hydrolysate frequently produced a burning sensation along the vein which was occasionally followed by venous thrombosis. The authors do not state whether similar occurrences were observed with the enzymatic hydrolysate or with the amino acid mixture. Presumably the lack of such a statement means that thrombosis was not observed.

A study such as this of the practical limitations of oral and parenteral "amino acid" therapy has been needed, particularly with respect to food intake, though it is regretted that the study was limited to so few individuals and really to only one patient.

CAROTENE AND VITAMIN A VALUES IN THE AGED

The so-called normal values for hemoglobin and erythrocyte counts, as usually given, are not necessarily normal for persons past the age of 65 years (B. Newman and S. Gitlow, *Am. J. Med. Sci.* **205,** *677 (1943);* **206,** *622 (1943)*). In general, the thiamine concentration in the blood and its excretion level tend to be low, while the incidence of porphyrinuria tends to be high in the aged (H. A. Rafsky and B. Newman, *Gastroenterology* **1,** *737 (1943); Am. J. Digest. Dis.* **9,** *43 (1942)*). The exact reasons for these differences in values for the so-called normal adults and the aged are not known. H. A. Rafsky, B. Newman, and N. Jolliffe (*Gastroenterology* **8,** *612 (1947)*) have continued studies of the differences in normal values with some observations on carotene and vitamin A in 14 male and 15 female apparently normal subjects ranging in age from 69 to 83 years. Chemical analyses revealed that the diet contained from 0.51 to 1.1 mg. of thiamine, 0.92 to 1.78 mg. of riboflavin, 16 to 117 mg. of ascorbic acid, 2.5 to 10.9 mg. of iron, 6.9 to 13.1 mg. of niacin, 1500 to 11,800 U.S.P. units of carotene, and from 1300 to 5500 U.S.P. units of preformed vitamin A. The concentration of vitamin A and carotene in the blood serum was determined when the subjects were in the fasting state and normal values were considered to be more than 100 micrograms per 100 ml. of serum for carotene and more than 40 micrograms per 100 ml. of serum for vitamin A.

Values for carotene in the serum of the 29 patients under investigation ranged from 18 to 156 micrograms per 100 ml., the average value being 80 micrograms. The value for carotene for the males averaged 74.7 micrograms and for the females, 85 micrograms. In 65 per cent of the subjects carotene levels were less than 100 micrograms per 100 ml.; of these 19 subjects, 8 were females and 11 males. The concentration of vitamin A for the entire group ranged from 6 to 78 micrograms per 100 ml. of serum. The average value was 27.1 micrograms. The values for the males averaged 29.3 micrograms and for the females, 25 micrograms per 100 ml. of serum. In 82 per cent of the entire series the values for vitamin A were less than 40 micrograms per 100 ml. Fourteen of these 24 persons were females and 10 were males. In only 7 per cent of the series, values for both carotene and vitamin A were normal, while in 55 per cent both values were subnormal. Of the subjects who had normal concentrations of carotene, 89 per cent had a concentration of vitamin A which was below normal and 60 per cent of those with

normal values for vitamin A had subnormal values for carotene levels.

The authors considered various possible explanations for these results. Inadequate intake seems unlikely. Vitamin A and carotene apparently are absorbed in sufficient amounts from the intestinal tract since vitamin A tolerance tests on aged individuals are reported to show that a rise in concentration in the blood does occur after oral administration of vitamin A. It is possible that these vitamins may not be stored in the liver, hence adequate concentrations in the blood are not maintained. It is also possible that vitamin A is not synthesized at the normal rate from the carotene that is ingested with the food. The authors believe this latter explanation is most likely and that the low concentration in the blood would point to diminution of hepatic function in subjects of advanced age. Some published data indicate that a large percentage of apparently healthy individuals past the age of 65 years have abnormal hepatic function, as measured by various tests for liver function. However, data of this type are extremely difficult to interpret.

Another possible explanation might be that so-called normal values given in the literature, which for the most part are based on studies on young adults or children, do not apply to the aged individual. Certainly these are interesting studies and future investigations along these lines should be fruitful.

PERSONALITY CHANGES IN MAN FOLLOWING RESTRICTION OF THE VITAMIN B-COMPLEX

The frequent complaints of decreased appetite, easy fatigability, lack of "pep" and of ambition, nervousness, and irritability are far from being specific signs of an inadequate vitamin intake. On the other hand, the early signs of endemic vitamin deficiencies, especially pellagra (T. D. Spies, J. Bradley, M. Rosenbaum, and J. R. Knott, *Res. Pub. Assn. Nerv. Ment. Dis.* **22,** *122 (1943)*), and the complaints of subjects on experimentally deficient dietary regimens indicate that these changes do occur in connection with restricted intakes of the B-complex vitamins (N. Jolliffe, R. Goodhart, J. Gennis, and J. K. Cline, *Am. J. Med. Sci.* **198,** *198 (1939);* R. D. Williams, H. L. Mason, R. M. Wilder, and B. F. Smith, *Arch. Int. Med.* **66,** *785 (1940);* K. O. Elsom, F. H. Lewy, and G. W. Heublein, *Am. J. Med. Sci.* **200,** *757 (1940);* E. E. Foltz, C. J. Barborka, and A. C. Ivy, *Gastroenterology* **2,** *323 (1944)*).

In addition to the complaints concerned with the more "physical" aspects, such as anorexia, thiamine deficiency has been reported to result in personality disturbances: irritability and moodiness, quarrelsomeness, lack of cooperation, restriction of activity, mental depression, and vague fears (R. D. Williams, H. L. Mason, B. F. Smith, and R. M. Wilder, *Arch. Int. Med.* **69,** *721 (1942)*). Because the subjects in the above study previously had been under psychiatric treatment the interpretation of the results is controversial. To obviate this difficulty, similar well controlled studies with normal subjects were indicated. The use of quantitative methods for the evaluation of psychologic variables further emphasized the importance of such work.

These considerations were incorporated in an experiment performed in the Laboratory of Physiological Hygiene at the University of Minnesota (J. Brozek, H. Guetzkow, and A. Keys, *Psychosomat. Med.* **8,** *98 (1946)*). The physiologic, biochemical, and dietetic aspects of the experiment have been reported in detail (A. Keys *et al., Am. J. Physiol.* **144,** *5 (1945)*).

The experiment consisted of four con-

secutive periods: (1) Standardization period of forty-one days. The subjects became adjusted to the laboratory routine, and their physical and psychologic characteristics were determined. (2) Partial restriction period of one hundred and sixty-one days. The dietary vitamin content was restricted to 0.18 mg. of thiamine, 0.29 mg. of riboflavin, and 3.7 mg. of niacin per 1000 calories. This represented about one third of the allowance recommended by the National Research Council for thiamine and riboflavin and two-thirds for niacin. The diet provided an average of 3300 calories. (3) Acute deficiency period of twenty-three days. The diet was essentially free of these vitamins. (4) Supplementation period during which to the deficient diet of the preceding period were added 10 mg. of thiamine for five days and 5 mg. of thiamine for the following five days.

Four of the normal young men ("control group") received the diet plus vitamin supplements which brought the intake of thiamine, riboflavin, and niacin up to that recommended in 1943 by the National Research Council. The other 4 men ("restricted group") were given placebos, identical in size and number to the vitamin capsules.

Different aspects of personality were examined by means of the following technics: (a) clinical observations; (b) subjects' reports on the state of their well-being as well as evaluation of their fellow subjects (self-ratings and inter-subject ratings); (c) detailed questionnaires on the physical and emotional state (Minnesota Multiphasic Personality Inventory); (d) an evaluation of the individual's imagination (Rorschach Inkblot test); and (e) performance under conditions designed to reproduce, in miniature, various stresses likely to be encountered in real life (Cattell's Miniature Situations personality test).

During the period of partial restriction no alterations in personality were observed clinically. Self-ratings and inter-subject ratings gave no evidence of a change in the well-being or in the social and emotional adjustment of the subjects. The findings of the Minnesota Multiphasic Personality Inventory were also negative. The Rorschach test given at the end of the partial restriction period showed slight changes in 3 out of 4 "restricted" subjects, interpreted as a decrease in spontaneity and presence of emotional tension. Cattell's test of Miniature Situations indicated a slight but statistically nonsignificant increase in one characteristic of the performance ("emotionality" score). All of these changes were minimal.

During the period of acute deficiency of thiamine, riboflavin, and niacin profound changes were found both clinically and by means of the standardized testing technics. An outstanding characteristic of this period was the apathy of the experimental subjects and their disinclination to spontaneous mental or physical activity. The self-ratings as well as the inter-subject ratings showed a sharp rise in the "neurasthenic" symptoms, the most prominent being loss of appetite, tiredness, sluggishness, and "blueness." In the Minnesota Multiphasic Personality Inventory the scores on the scales for the "psychoneurotic triad" (depression, hypochondriasis, and hysteria) showed a statistically and clinically significant departure from normal. The Rorschach records suggested an increase in the "conscious control" over behavior. Cattell's Miniature Situations test indicated some rise in "emotionality" and "timidity" scores; on the contrary, the subjects showed normal scores for those aspects of performance which distinguish psychotics from normals. In summary, the vitamin deficiency produced a picture of "psychoneurosis," with no deep (psychotic) personality alterations. Supplementation of the deficient diet with thiamine resulted in a rapid recovery.

Similar results were obtained in an experiment carried out at the Army Medical

Nutrition Laboratory in Chicago (C. R. Henderson *et al.*, *Am. J. Med. Sci.* **213,** *488 (1947)*). The experimental diet provided daily an estimated 3300 calories and contained 0.15 mg. of thiamine, 0.09 mg. of riboflavin, and 1.76 mg. of niacin per 1000 calories. Following the standardization period of twelve weeks, all 7 subjects were placed on the experimental diet for five weeks; the significance of this period in the general design of the experiment is not clear. At the end of the fifth week, 2 subjects were selected as controls and received crystalline supplements of the B-complex vitamins in amounts which raised the intake to equal or exceed the levels of the pre-experimental diet (1.44 mg. thiamine, 1.84 mg. riboflavin, and 15.60 mg. niacin daily). The scores on the depression, hysteria, and hypochondriasis scale of the Minnesota Multiphasic Inventory showed a rise in all 5 restricted subjects after one hundred and five days on the low vitamin diet. The experimental design of the supplementation period was not uniform for all the men and the results cannot be summarized with ease. In general, the supplementation of diet with thiamine and other components of the vitamin B-complex resulted in a reversal of the trend in the scores for some of the subjects but the pre-experimental, normal level was reached only after three weeks on an unrestricted diet.

These studies indicate that the field of human nutritional research may be significantly broadened by studying the effects of diet on psychologic aspects of "fitness," including personality, by means of quantitative methods, standardized on normal individuals and evaluated against a background of clinical psychiatric experience.

THE CHEMICAL EVOLUTION OF VISION

"Ah, Sunflower! weary of time,
Who countest the steps of the sun,"

Thus did Blake one hundred and fifty years ago personify the sunflower and its phototropism. The close analogies between the vision of animals and the "vision" of plants which results in phototropisms have been summarized by G. Wald (*Harvey Lectures* **41,** *117 (1945–46)*) who presents evidence that an actual evolutionary relationship exists between the photoreceptivity of plants and animals.

It is well established that vision in the higher animals is consequent to the absorption of light by several pigments which are composed of a protein and a carotenoid. The two pigments which have received most study are rhodopsin, which contains retinene$_1$, a derivative—possibly the aldehyde—of vitamin A_1, and porphyropsin which contains retinene$_2$, a derivative of vitamin A_2. The latter pigment is confined chiefly to those fish which spawn in fresh water and to amphibia before metamorphosis. Wald's review and previous reports may be consulted for a fuller description of this interesting subject.

Carotenoids are widely distributed in plant material and it has been difficult to discover any metabolic function for these pigments. Instead, they seem to be responsible for photoreception. Photosynthesis and phototropism were shown early to be dissociated from each other. Thus, yellow light is active in producing photosynthesis but without phototropic effect, whereas, blue light which has but little photosynthetic effect, is strongly phototropic. Obviously, light to have an effect must be absorbed; hence a yellow absorbing pigment, i.e., chlorophyll, is involved in photosynthesis and a blue absorbing pigment or pigments must be concerned in phototropism. When the quantitative efficacy of different wavelengths of light in producing bending is plotted against

the wavelength, the resultant curve almost exactly coincides with the absorption spectrums of the carotenoids extractable from that species of plant. For example, the coleoptile of the oat seedling responds more readily to light of wavelengths of 440 and 475 millimicrons than to light of intermediate wavelengths, and the carotenoids extractable from the coleoptile (chiefly leaf xanthophyll) show absorption maxima at these same two wavelengths. On the other hand, the mold, Pilobolus, which contains chiefly beta-carotene with absorption maxima shifted toward longer wavelengths, has its "photoresponse" spectrum similarly shifted toward longer wavelengths.

The green flagellates, which may be regarded as either plants or animals, possess a photosensitive eye spot in which the carotenoid astaxanthin appears to be localized. This carotenoid has only one absorption maximum which falls between 470 and 492 millimicrons depending on the solvent. The photoresponse curve for these organisms similarly shows a single peak which occurs at various wavelengths from 473 to 534 millimicrons, depending on the species. Wald ascribes the shifts in the response peak to different physical states of the pigment and to possible protein combinations. This appears entirely reasonable. The well known change in the color of lobsters from green to red on boiling is known to result from the conversion of a green protein-astaxanthin combination to the red of free astaxanthin.

There is evidence that the photosensitivity of some of the eyeless invertebrates—hydroids, certain worms and clams—is dependent on carotenoids but the studies are less complete than with the plants and green flagellates. Here lies an important gap in the chemical evolutionary argument. By the time the first image-forming eyes are reached in the arthropods the photoreceptor function has been taken over by derivatives of vitamin A rather than the plant carotenoids. The field of chemical evolution would be furthered by studies covering this gap.

Although photoreception in higher animals is performed by derivatives of vitamin A, the plant carotenoids appear to have at least a minor role in the cone vision of certain birds and mammals. Both xanthophylls and a carotene are present in chicken cones and are believed to function as filter materials in color vision. Similarly, a xanthophyll is present in the macula lutea of primates and is thought to sharpen central vision by reducing the effects of chromatic aberration. The further study of chemical evolution in this and other areas is of interest for its own sake and is likely to contribute a better understanding of mammalian function.

TOCOPHEROLS AND THE PREVENTION OF GASTRIC ULCERS PRODUCED BY DIETARY MEANS

A number of nutritional factors are concerned with the maintenance of an intact gastric mucosa in experimental animals. From various sources information has accumulated which demonstrates that ulceration of the gastric mucosa may occur in deficiencies of protein, vitamin A, cystine, choline, riboflavin, pyridoxine, pantothenic acid, and calcium (*Nutrition Reviews* **1,** *396 (1943);* **2,** *13 (1944);* **4,** *278 (1946)*). Recent reports indicate that gastric ulcers are not the result of these deficiencies themselves, but occur when the essential nutrient is supplied to deficient animals. Furthermore, tocopherols will prevent the development of mucosal lesions under such conditions. J. L. Jensen (*Science* **103,** *586 (1946)*) found ulcers of the forestomach in 67 per cent of a group of rats maintained for forty-eight days on a diet free of vitamin A to which was

added a daily supplement of 0.57 microgram of vitamin A. No gastric ulcers occurred in rats receiving the same diet supplemented daily with 0.57 microgram of vitamin A plus 0.5 mg. d, alpha-tocopherol. Since the gain in body weight was greater in those animals receiving the tocopherol it seemed probable that tocopherol was effective in preventing the formation of gastric ulcers by means of its vitamin A-sparing action.

P. L. Harris, E. L. Hove, M. Mellott, and K. Hickman (*Proc. Soc. Exp. Biol. Med.* **64,** *273 (1947)*) investigated further the role of tocopherol in preventing the dietary production of gastric ulcers. Weanling rats placed on a diet deficient in vitamins A and E for two weeks were then kept on the same diet for an additional seven weeks but were given 300 units of vitamin A daily, i.e., realimented. In addition, the diets of half of the group of rats were supplemented with 0.5 mg. d, alpha-tocopherol daily. At the end of the seven week period, 50 per cent of those on the diet without tocopherol had ulcers of the forestomach, while no ulcers were found in the stomachs of animals receiving alpha-tocopherol. It was evident, therefore, that the provision of large quantities of vitamin A does not prevent gastric ulcer formation.

An attempt was made to determine the effect of varying types and amounts of fat in the diet. The diet used was the U.S.P. vitamin A test diet. The fat content was varied as follows: 5 per cent olive oil, 10 per cent olive oil, 5 per cent stripped (vitamin E-free) corn oil, 10 per cent stripped corn oil, and low fat (0.3 to 0.4 per cent). Groups of weanling rats were placed on these diets. In addition, half of the rats on the low fat diet were supplied with 100 mg. of sesame oil daily to supply adequate linoleic acid. The various groups were observed until evidences of vitamin A depletion were apparent. At that time there were no gross lesions of the gastric mucosa found in 4 rats which were killed. The remaining rats in each group were provided with daily supplements of either 2 or 30 units of vitamin A and half of the animals in each group also received 0.5 mg. of alpha-tocopherol daily. At the end of seven weeks vitamin A in the doses administered did not prevent the occurrence of mucosal changes but when tocopherol was included in the daily supplement, the incidence of gastric ulcers was markedly reduced in all groups, except in rats on the low fat diet either with or without sesame oil. Since the provision of 30 units of vitamin A daily did not prevent the occurrence of lesions in the gastric mucosa, it seemed unlikely that the effect of tocopherol could be ascribed to its vitamin A-sparing action. However, there was an indication that tocopherol was effective through some action taking place in the gastrointestinal tract, since tocopheryl phosphate injected parenterally did not reduce the incidence of gastric ulcers when administered under the conditions of this experiment.

Evidence was obtained to indicate that the amount of fat in the diet is a factor in the prevention of rumen lesions. There was a high incidence and greatest severity of lesions in rats on the low fat diet. Mucosal defects occurred frequently in animals on diets containing 5 per cent olive oil or stripped corn oil but were absent or markedly reduced in number when a 10 per cent level of intake of fat was provided even in rats not receiving supplements of tocopherol.

Other groups of weanling rats were placed on diets free of fat to assess the effect of a diet deficient in essential fatty acids. After seven weeks' depletion no evidences of lesions in the gastric mucosa were found. However, the addition of 20 mg. of ethyl linoleate to the daily diet brought about a high incidence of lesions. This was prevented by a daily dietary supplement of tocopherol.

Harris and his co-workers then set out to determine whether the administration of alpha-tocopherol would prevent the occurrence of gastric ulcers in rats deficient in pyri-

doxine. After six weeks' depletion on a pyridoxine deficient diet, groups of rats were provided with daily supplements of pyridoxine varying from 0 to 8.0 mg. of the free base. Half of each group also received 1.0 mg. of alpha-tocopherol daily. After six weeks pyridoxine deficiency did not result in rumen lesions. With daily supplements of from 1.0 to 4.0 mg. of pyridoxine, lesions did occur and were prevented by the inclusion of tocopherol in the diet.

The authors point out that severe tocopherol deficiency *per se* is not accompanied by the production of gastric ulcers and that single deficiencies of vitamin A, linolenic acid, and pyridoxine do not produce such lesions. When the missing factors are provided to rats deficient in these latter three substances ulcers occur, which are prevented by supplying tocopherol. They consider that the epithelium of the forestomach becomes abnormal in these deficiency states, food intake decreases, weight gain ceases, and gastric secretion of pepsin and hydrochloric acid probably diminishes. When the missing factor is administered the food intake increases and weight is gained. It is their opinion that the functionally abnormal epithelium cannot cope with the increased amounts of pepsin and hydrochloric acid secreted and ulcers result. The explanation is offered that tocopherols may prevent this process of erosion by specifically inhibiting enzyme activity or by increasing epithelial resistance through greater mucus secretion or by improving the blood supply to the mucosa. These are, however, speculations which need to be supported or abandoned on the basis of further work.

There may be in these observations a means of study of the mode of action of tocopherol and the functions it controls in the digestive process. At this time there is no reason to assume that such factors are important in the causation of peptic ulcers in man.

THE "PROTEIN EFFECT" OF GLYCINE

Several years ago A. Mirski, I. Rosenbaum, L. Stein, and E. Wertheimer (*J. Physiol.* **92,** *48 (1938)*) presented evidence that the glycogen reserves of the liver which had been laid down on a high protein diet behaved differently than those glycogen reserves which were deposited on a diet predominantly of carbohydrate. These workers found that nonfasting animals which had been maintained on a diet high in carbohydrate and containing 20 per cent protein had an average liver glycogen content of approximately 4 per cent. Similar animals fed a diet containing 70 to 90 per cent casein had a mean nonfasting liver glycogen content of 1.12 to 1.38 per cent. Following a twenty-four or forty-eight hour fast, however, a higher glycogen content was encountered in those animals which had received the diet containing 70 per cent casein. The differences were striking. Thus, at the end of a twenty-four hour fast the animals previously fed 20 per cent casein had liver glycogen levels of 0.08 per cent, those which had received 70 per cent casein showed levels of 1.42 per cent, and those receiving 90 per cent casein, 1.00 per cent. Interestingly enough, after seventy-two hours of fasting the liver glycogen level of animals fed 20 and 70 per cent casein respectively were 1.22 and 1.15 per cent. These latter results were attributed to the protein-rich diet of the fasting animal as revealed by the determinations of nitrogen excretion.

The increased stability of the liver glycogen levels of animals pre-fed on high protein diets was demonstrated to be due to some factor other than the resistance of the glycogen to glycogenolysis. Thus, in work experiments in which the rats were made to swim for one to two hours and killed immediately thereafter the mean liver glycogen

content of the animals fed 70 per cent casein was actually lower than that of animals fed 20 per cent casein. Within four hours after exercise, however, more glycogen had accumulated in those animals which had previously been fed on the diet containing 70 per cent casein. These and similar experiments employing other methods of increasing glycogenolysis led Mirski and co-workers to postulate that regeneration of glycogen through glyconeogenesis during a recovery period is far more pronounced in animals which have previously been maintained on a diet rich in protein than in animals pre-fed a diet rich in carbohydrate. These effects of high protein feeding manifested by greater stability of the glycogen level in the liver are frequently referred to as the "protein effect." Fifty per cent casein in the diet was the minimal level suitable for demonstrating this effect and 70 per cent casein was the optimal concentration for showing the effect.

W. R. Todd, J. M. Barnes, and L. Cunningham (*Arch. Biochem.* **13,** *261* (*1947*)) have now demonstrated that pre-feeding ats with a diet containing 10 or 15 per cent glycine for forty-eight hours is followed by a "protein effect" at the end of twenty-four hours of fasting. For these studies male rats of the Sprague-Dawley strain were fed a diet containing 16 per cent casein and 10 per cent brewers' yeast as sources of protein. When amino acids were incorporated they replaced a like amount of dextrin. The magnitude of the effects observed after feeding with glycine is illustrated by the following mean liver glycogen levels at the conclusion of twenty-four hours of fasting: animals pre-fed on the basal diet, 10 per cent glycine, or 15 per cent glycine, 0.33, 1.10, 1.17 respectively; animals pre-fed on a diet containing 18 per cent dl-alanine showed fasting liver glycogen contents of 0.56. It is further stated that l-leucine and l-glutamic acid do not show this effect when fed at levels equimolecular with 10 per cent glycine. Adrenalectomy, which Mirski *et al.* showed eliminates the "protein effect," also resulted in loss of this action by glycine. This phenomenon appears to be worthy of additional studies in relation to the physiologic effects of high protein diets.

FOLIC ACID (PTEROYLGLUTAMIC ACID) AND LACTATION

Some of the data bearing on the existence of a factor necessary for lactation in rats have been reviewed in this journal (*Nutrition Reviews* **5,** *221* (*1947*)). The criteria for a lack of this factor or factors have been the proportion of young weaned, the weight loss of females during lactation, and the smaller size of the young weaned from females lacking in this factor. M. M. Nelson and H. M. Evans (*Arch. Biochem.* **13,** *265* (*1947*)) have shown that the maternal leucocytes at weaning are considerably reduced when mothers are placed at parturition on the diet lacking this factor(s). Thus, the maternal leucocytes at weaning averaged 10,850 per cubic millimeter in 43 rats which were maintained on the stock diet but numbered 4350 in 42 rats on the basal purified deficient diet. Not only was there a reduction in the total white blood cells, but there was a relatively greater decrease in the polymorphonuclear leucocytes from a mean of 30 per cent of the total white cells in the mothers on the complete stock diet to 18 per cent in those maintained on the deficient diet. The workers present the average figures along with the ranges of values. These ranges indicate a definite overlapping of the data. Although no significance tests of these values are included in the report, the magnitude of the differences would seem to indicate true differences.

The addition of various levels of synthetic folic acid (pteroylglutamic acid—PGA) to the deficient diet was followed by a slightly greater average weaning weight of the young, a mean increase in weight of the mother

during lactation, a greater average total maternal leucocyte count at weaning, and increased percentage of polymorphonuclear leucocytes above that of mothers maintained on the basal diet alone. The maximal effect of the PGA on the body weight and circulating leucocytes of the lactating mother apparently occurred at the lowest level at which the vitamin was fed (110 micrograms per 100 g. of diet).

The addition to the deficient diet of a liver eluate powder which contained Lactobacillus casei factor equivalent to the same amounts of PGA as fed in the above described experiments resulted in greater average weaning weight of the young, greater mean increase in weight of the mother during lactation, and larger average total leucocyte counts than did feeding equivalent amounts of PGA.

These investigators conclude that while pteroylglutamic acid is one of the factors required by the rat during lactation, the liver eluate powder contains an unknown factor(s) which is also necessary during this period. A daily intake of 33 micrograms or less of PGA is required for maximal effects on maternal body weight and circulating leucocytes. Between 33 and 81 micrograms per day is suggested as the requirement for the maximal effect on the weaning weights of the young. The data presented appear to justify the conclusions reached. In view of the wide ranges indicated the data might be made more convincing, however, if they were presented in a manner which would permit their statistical evaluation.

OPTIC NEUROPATHY IN PRISONERS OF WAR

An increasing number of reports emphasize the occurrence of neuropathy among prisoners of war existing upon starvation rations (*Nutrition Reviews* **4,** *56, 208, 253 (1946)*). The clinical picture of this neuropathy is somewhat variable and in many instances those workers who have reported observations of the syndrome have been hesitant to ascribe it to malnutrition. Many of the reports describe ocular symptoms accompanying more generalized evidences of neurologic damages. The papers of J. G. Reed (*Tr. Roy. Soc. Trop. Med. Hyg.* **40,** *411 (1947)*) and P. G. Bell and J. C. O'Neill (*Canad. Med. Assn. J.* **56,** *475 (1947)*) deal primarily with the ocular manifestations and are more or less representative of the descriptions of the condition. Shortage of personnel and investigative supplies as well as the conditions existing in the prison camps have made it impossible for any group to study the ocular neuropathy in detail and, therefore, to give a unified picture of the pathology and etiology of the disease.

Reed (*loc. cit.*) described the clinical syndrome as he observed it among British service men imprisoned by the Japanese at Palembang, Sumatra. Eighteen months following imprisonment, 126 of 490 men, or 26 per cent of the group, complained of visual disturbances. These men had been on a diet deficient in protein, fat, and the B-group of vitamins for eighteen months. Gross deficiency diseases had not appeared—only one case of beriberi had been observed and no pellagra had developed. After thirty-three months of imprisonment, 32 per cent of the men in the camp complained of visual disorders. However, these ocular complaints were more conspicuous prior to the appearance of full-blown beriberi and pellagra in the camp population than after these diseases become common.

The ocular symptoms were usually gradual in onset requiring weeks to months for full development. In most instances both eyes were affected. They were characterized by extreme sensitivity to light, blurring of vision, and impairment of sight for distant

objects. Pain and injection of the cornea and excessive lacrimation were common. After a few weeks or months the pain and irritation ceased or decreased, but the defective vision showed little, if any, improvement. The defective vision did not improve between 1943 and 1945 in the majority of the severe cases. Night blindness was not a part of the picture. Ophthalmoscopic examinations were not routinely made, but in a few cases observed after the acute phase and exhibiting residual defects it was stated that temporal pallor of the discs was a constant feature.

The accompanying signs of malnutrition in the individuals manifesting the eye syndrome were: angular stomatitis, 45 per cent; glossitis, 38 per cent; angular stomatitis and glossitis, 23 per cent; generalized stomatitis, 1.6 per cent; cheilosis, 4 per cent; and neuritis or paresthesias, 25 per cent. It is stated that the incidence of these signs was higher among those suffering from the ocular syndrome than among the remainder of the prisoners. The general physical condition of the patients suffering with the syndrome was classified as good in 50 per cent and poor in 12 per cent of the cases. The attempts at therapy shed little light on the cause of this outbreak.

Reed concludes that this condition may be of toxic infectious origin, a manifestation of a dietary deficiency, or due to a combination of these. He rules out the usual toxic causes of such eye conditions in his series, but notes that the possibility of food toxins contained in Dioscorea (a tuber) and some of the cyanogen-containing foodstuffs, such as the cassava, must be considered. The etiologic role of infections could not be ruled out inasmuch as skin diseases were common among the men in the camp. The final impression of this worker was that these cases were examples of ariboflavinosis, possibly superimposed on an infective factor. He surmised that the lesion was most probably a retrobulbar neuritis.

One hundred fifteen cases of optic neuropathy, which occurred among 560 men of the Winnipeg Grenadiers who returned after four years of captivity in Hong Kong, were studied by Bell and O'Neill (*loc. cit.*). During imprisonment these men had subsisted on a diet composed largely of polished rice "with occasional supplies of half rotten fish or meat, soya beans, a little flour and sugar, and a fair quantity of mixed vegetables." An unidentified weed, described as particularly vile tasting, was sometimes eaten in limited quantities. The average weight loss per man was about 35 to 40 pounds during the first few months of imprisonment. During the period of captivity up to 60 per cent of the men experienced some visual disturbances. The development of these disturbances followed closely the pattern described in the British prisoners in Sumatra (Reed, *loc. cit.*). Although these ocular symptoms developed while the subjects were subsisting on a grossly deficient diet, the concurrence of beriberi, pellagra, dysentery, malaria, jaundice, or diphtheria was not essential to the development of the neuropathy. One orderly stated that the visual disturbances occurred while he was taking injections of thiamine plus "vitamin wafers."

Bell and O'Neill state that prisoners of war in Europe, who were also starved and suffered weight losses of 40 to 50 pounds, did not, in their experience, suffer from the ocular syndrome or beriberi. These workers raised the question whether this syndrome might be due to B-vitamin deficiency or, possibly, to some toxic factor present in rice or moldy rice.

Objectively, the residual defects which were observed in the men studied by Bell and O'Neill consisted only of pallor of the temporal quadrant of the disc. Macular vision was destroyed in many cases with the peripheral fields of vision remaining fairly normal. Determinations of the visual field indicated the presence of variously sized blind spots or scotoma which were invariably in the central field of vision. These workers apply the term optic atrophy to the lesion. Despite therapy with high-protein-high-

calory diets and multivitamin preparations, little, if any, change occurred during a period of approximately a year.

These, and the several other clinical reports which have appeared, leave little room to question that this ocular neuropathy is a definite syndrome which has resulted in permanent partial blindness in from 20 to 30 per cent of many of those groups held prisoners by the Japanese. The evidence is highly suggestive but inconclusive that the etiology of the condition is, at least in part, the malnutrition. Definite association with the recognized deficiency diseases, however, does not appear. It is possible that some insight could be realized on the etiology of this syndrome by means of animal experiments in which the dietaries were patterned after those consumed by prisoners in camps with a high incidence of ocular neuropathy.

MILK SECRETION OF VITAMIN D IN THE RAT

No report has been made in the past on the ability of the lactating female rat to secrete ingested vitamin D in the milk and thus make it available to her young. Several investigators have reported this ability to be limited in human beings, and the same has been found to be true for the cow and the ewe (see *Nutrition Reviews* **4**, *157 (1946)*). An accident which occurred in a rat colony maintained for raising animals for vitamin D assay has been described by J. Embleton and A. J. Collings (*Nature* **159**, *340 (1947)*), because it gives some indication of this ability in the rat.

The diet of the stock colony of rats was described by K. H. Coward, M. R. Cambden, and E. M. Lee (*Biochem. J.* **26**, *679 (1932)*), and it contains 10 per cent full cream dried milk powder. Litters of young intended for vitamin D tests are weaned at 21 to 23 days of age and fed the stock diet until they weigh 55 to 65 g., which takes about ten days. If they are then given the Steenbock rachitogenic diet no. 2965 for a preparatory period of three weeks, they develop severe rickets and are suitable for use in the assay of vitamin D. A further period of ten days on the same diet with the addition of a small dose of the standard or unknown vitamin D-containing substance results in formation of a line of healing in the metaphyses of the ulnas and radiuses, with well defined areas of uncalcified cartilage above and below it.

In August 1945 it was noted that healing as shown by the line test became very high, and by the end of September little rickets developed in the rats even after four and a half weeks on the rachitogenic diet. The young rats grew well and showed obvious signs of well-being when fed on the rachitogenic diet. Moreover, the colony maintained its reproduction rate unusually well toward the end of 1945, instead of showing the usual seasonal drop in the number of litters produced in the last months of the year. Toward the end of December it was learned that the producers of the dried milk had been supplying milk fortified with vitamin D since the beginning of August, without having informed the laboratory of the change.

The change back to unfortified milk was made at the beginning of January 1946 and the reappearance of rickets in young rats fed on the rachitogenic diet was followed by using fresh litters every week, and examining them after a suitable period on the diet, by application of the line test. Mild rickets first began to develop about the end of February, and as time passed the rickets became more severe until at the beginning of May it was sufficiently severe so that the rats could again be used for vitamin D assays after the usual three week preparatory period.

These observations indicate that during the period January to April 1946 the young

rats which did not develop rickets after the usual treatment must have contained vitamin D within their bodies, which they had obtained through the placenta or milk of their mothers and had conserved within their bodies. Similarly, this store was being obtained from a mother who was not, after the end of 1945, receiving sufficient vitamin D to maintain her own vitamin D store at the unusually high level to which it had been raised by a five month supply of fortified milk to the colony, during which it was estimated that each female received on an average 15 I.U. of vitamin D daily for one hundred and forty-two days, or a total of 2130 I.U. from the milk, plus an unknown but small amount from the rest of the food.

There was no attempt to examine the stock rats to determine how large their stores might have been; the only information is that these stores were exhausted four months after the end of the three month period of feeding on the enriched diet. During these four months the females raised, on the average, 2 litters each, presumably giving the young rats decreasing amounts of vitamin D as the time went on.

That the female stock rat would benefit by receiving more vitamin D than the stock diet usually contains is indicated by the fact already mentioned that the number of litters produced in the stock colony did not fall off during the last months of 1945, as it had always done previously at this season. The stock diet was of course purposely kept as low as possible in vitamin D, so that the stock could be maintained.

METABOLIC CHANGES IN DIABETIC ACIDOSIS

Although the metabolic changes in diabetic acidosis have been studied extensively, many clinical features remain unexplained. H. E. Martin and M. Wertman (*J. Clin. Invest.* **26,** *217 (1947)*) have added some important data by serial measurements of the serum potassium, magnesium, and calcium concentrations in 14 patients with diabetic acidosis (carbon-dioxide combining power below 9 milliequivalents per liter). Three of the patients were comatose on admission, 3 semicomatose, and 8 conscious; one patient, admitted in coma, died. In addition to the electrolytes already mentioned, serum sodium, total protein, blood sugar, carbon-dioxide combining power, and nonprotein nitrogen were determined on every patient; pH, albumin-globulin fractionation, and serum inorganic phosphorus determinations were made on a few patients.

In addition to the usual findings of acidosis, hyperglycemia, and hemoconcentration, fluctuations in the serum potassium, magnesium, and phosphorus concentrations were observed during the course of therapy. The serum potassium was above 6 milliequivalents per liter in the 5 patients on whom the determination was made on admission, the highest being 10.3. During therapy low serum potassium occurred usually within the first twenty-four hours and frequently fell to very low values (1.9 and 2.18 milliequivalents per liter). The potassium concentrations returned to normal levels usually within two or three days.

The serum magnesium was determined on 5 patients on admission, was slightly elevated in 2, normal in 2, and slightly below normal in one. In almost all the patients the serum magnesium fell at some time to abnormally low values, usually during the first twenty-four hours after admission, and then gradually rose, although more slowly than was the case with serum potassium. There was a rough correlation between the state of consciousness on admission and the serum magnesium concentrations.

Serum calcium concentrations were generally normal throughout, although it was thought that the total circulating calcium

was probably reduced due to the hemoconcentration.

The serum sodium concentration was either normal or somewhat low on admission, but soon after therapy was begun came into the normal range. In a few instances, high values during therapy are recorded and are probably associated with the administration of normal saline.

Inorganic phosphate determinations were made on only 2 patients and in both the changes correlated with the serum potassium and blood sugar levels during therapy, which in all patients consisted in varying amounts of normal saline, one-sixth molar sodium lactate, 5 per cent glucose, plasma, and insulin.

The authors discuss the interpretation of their data, compare their results with those of others, and in particular point out the similarity between the marked muscle weakness shown by several of the patients and that seen in familial periodic paralysis, another condition associated with low serum potassium concentration. J. W. Holler (*J. Am. Med. Assn.* **131,** *1186 (1946)* and M. Frenkel, J. Groen, and A. F. Willebrands (*Nederl. Tijdschr. v. geneesk. (in press, 1947)*) have described patients who developed a similar syndrome during therapy for diabetic acidosis or coma. The latter authors noted, in addition to muscular weakness and "lower" respiratory paralysis, a high pulse pressure, cardiac enlargement, and a high venous pressure. Therapy with potassium produced dramatic improvement.

In interpreting the changes in serum potassium concentration, Martin and Wertman mention possible changes in urinary excretion or use of the potassium in carbohydrate metabolism. As magnesium is, like potassium, nitrogen, and phosphorus, an important intracellular component it is not surprising to find that the movement of magnesium follows that of the other three. However, as the authors point out, until intracellular concentrations of these substances are known it will be difficult to interpret adequately changes in serum concentration. However, D. Atchley *et al.* (*J. Clin. Invest.* **12,** *297 (1933)*) showed that with the negative nitrogen balance which occurred in diabetic patients following withdrawal of insulin, a negative phosphorus and potassium balance could be demonstrated as well. One might, by combining the data of Martin and Wertman with that of Atchley *et al.*, assume that there is an intracellular depletion of potassium with increased serum concentration and loss in the urine during the period of insulin lack. On supplying insulin, the reverse would be true, but because of the potassium loss in the urine a body deficit exists, thus reducing serum potassium concentration. If this interpretation is correct, the administration of potassium as therapy is called for, as the authors suggest.

The warning given by the authors against the administration of potassium to diabetic patients in shock is well taken and, in fact, one might even say that potassium should be administered only very cautiously, or not at all, until adequate urine output has been established. Several reports of dangerously high blood potassium concentrations have been published and have been added to and reviewed by C. A. Finch, C. G. Sawyer, and J. M. Flynn (*Am. J. Med.* **1,** *337 (1946)*). It is especially noteworthy that a flaccid paralysis resembling that seen with low serum potassium may result from potassium intoxication as well.

DELAYED AMINO ACID SUPPLEMENTATION IN RATS

The utilization of large amounts of amino acid mixtures injected intravenously or administered orally may present different problems from those encountered in the slower digestion and absorption of the amino acids in dietary proteins. The problems encoun-

tered might be especially different if the ratio of the amino acids in the mixture was not the exact ratio needed for the building or maintenance of body proteins or if some of the essential amino acids were lacking. The fate of such an inadequate mixture might be considered in terms of at least three possibilities: (1) storage of the amino acids as such, (2) storage of the amino acids in the form of osmotically less active peptides, and (3) irreversible metabolism of the amino acids and their resultant loss for protein synthesis. The first two possibilities were tested by E. Geiger ("Physiology of the Utilization of Dietary Amino Acid Mixtures," *Dissertation, University of Southern California, Los Angeles (1947)*). In those experiments no transitory storage of incomplete amino acid mixtures in the form of either amino acids or peptides could be detected. In view of these findings an indirect approach to the third possibility was tested using rats as the experimental animal (E. Geiger, *J. Nutrition* **34,** *97 (1947)*). A diet containing an incomplete amino acid mixture was fed for twelve hours each day and then in the alternate twelve hours the rats were fed an identical diet with the exception that the incomplete amino acid mixture had been replaced by an appropriate amount of the amino acid or amino acids missing in the amino acid mixture. Thus in each twenty-four hour period the rats had access to an amino acid mixture which, if utilized efficiently, would constitute an adequate source of the essential amino acids. The distribution and amounts of carbohydrates, fats, vitamins, and salts available were the same over the entire twenty-four hour period. If the components of the amino acid mixture could be stored by the rats for even a few hours, protein synthesis and body growth would occur after the missing amino acid or acids were provided. On the other hand if the body were unable to store one or more of the components of the amino acid mixture until the addition of the missing ones, then only incomplete components would be available for protein anabolism.

Mackerel proteins which have been demonstrated to be of higher biologic value than casein (*Nutrition Reviews* **4,** *189 (1946)*) were used as the basis for the preparation of an amino acid mixture deficient in tryptophane. This mixture was prepared from mackerel proteins by hydrolysis with sulfuric acid at 15 pounds pressure for eight hours. After removal of the sulfuric acid by barium hydroxide, the solution was concentrated under reduced pressure and spray dried. When fed at a level in the diet equivalent to 3 per cent nitrogen to young rats (as the sole source of protein), poor growth resulted. If this diet was supplemented continuously with 1 per cent l(−) tryptophane, normal growth was observed. When the diet containing 30 per cent of the tryptophane deficient amino acid mixture was fed at alternate twelve hour periods with a diet containing 6 per cent of l(−) tryptophane, practically no growth occurred during the experimental period. In an additional group of rats, access was allowed continually to both the above diets on a self-selection basis. For the first six to seven days the rats in this group did not grow any better than the alternate feeding group. After this period growth occurred at a normal rate indicating that the rats had in some way learned to eat from each jar at close enough intervals to permit supplementation of the incomplete amino acid mixture.

A lysine deficient amino acid mixture was prepared from casein by a nitrite procedure (M. S. Dunn and H. B. Lewis, *J. Biol. Chem.* **49,** *327 (1921)*). When a diet containing the nitrite-treated casein as the sole protein was fed to rats, a rapid weight loss was observed. Lysine supplementation did not result in a decreased weight loss and other experiments indicated that this lysine deficient amino acid mixture was toxic to growing rats. Therefore Geiger chose to study delayed amino acid supplementation by the use of zein which is a lysine deficient protein.

Since zein is also deficient in tryptophane, the control ration was supplemented with lysine and tryptophane. Even when these supplements were given simultaneously, the resultant growth was suboptimal as observed previously by H. A. Harris, A. Neuberger, and F. Sanger (*Biochem. J.* **37,** *508 (1943)*). The rats in the alternate feeding group received a diet containing zein plus an adequate supplement of tryptophane for twelve hours each day and an identical diet in which the zein and tryptophane were replaced by lysine for the alternate twelve hour period. The rats conspicuously preferred the diet containing the lysine but did not grow in the twenty-five day experimental period. An additional group of animals received a diet containing zein and lysine for twelve hours and a diet containing an adequate amount of tryptophane for the alternate twelve hours. Food consumption was good in both periods but again the rats did not grow. In a third experimental group the rats were allowed access to three diets, one containing zein, another lysine, and the other tryptophane. No efficient selection of food was demonstrated and again the rats did not grow.

In a similar series of experiments with methionine where an amino acid mixture deficient in methionine and tryptophane was prepared from casein by the formic acid-hydrogen peroxide procedure of G. Toennies (*J. Biol. Chem.* **145,** *667 (1942)*), rats were unable to utilize the amino acid mixture with the delayed supplementation of methionine. Thus in these experiments with rats the delayed supplementation of an incomplete amino acid mixture with the missing ones has been demonstrated to be ineffective in the case of tryptophane, lysine, and methionine.

Various suggestions that amino acids cannot be stored in the body have been presented under widespread differences in experimental procedure but this is the first clearcut demonstration of the dramatic effect that this inability to store protein constituents may have on protein synthesis and body growth. When amino acid mixtures are being prepared for therapeutic purposes, these results should be considered. Unfortunately our knowledge of how many amino acids are essential to the human being and in what ratio they are required under different circumstances of health and disease, growth, and maintenance are so limited that the most accurate calculation possible may result in estimates far from the true values. Since the data of W. M. Cox and A. J. Mueller (*Federation Proceedings* **3,** *56 (1944)*) demonstrated that less amino acids are required in the day for maintenance of nitrogen balance when the nonessential ones are provided simultaneously, calculations of therapeutic mixtures of amino acids may also have to consider the inclusion of additional amounts of certain essential amino acids for the synthesis of nonessential ones or the inclusion of nonessential amino acids in the mixture.

It would seem that the length of time necessary in the animal body to digest proteins and to absorb the resulting amino acids would reduce the destructive metabolism of components of one inadequate protein until the missing amino acids could be supplied from the digestion of other proteins. However, as a test of this hypothesis, an investigation along the lines of Geiger's experiments would be valuable using two diets each containing an incomplete protein, with the two proteins being selected for their ability to supplement each other if fed simultaneously in a diet at comparable levels. An investigation of the effect of a more rapid exchange of the two supplementary rations used by Geiger in his various experiments would also be of considerable interest as it is conceivable that there is a critical period which is fairly long but less than twelve hours during which supplementation must occur. It would be advisable in future experiments to have more than 2 animals in each group although in Geiger's experiments the growth response of the 2 rats in each experimental treatment duplicated each other very well.

NOTES

Letter to the Editor

Dear Sir:

It is unfortunate that, in your excellent article on "Present Knowledge of Vitamin-Enzyme Relationships" (*Nutrition Reviews* **5,** *194* (*1947*)), you did not include the work of W. W. Umbreit, W. A. Wood, and I. C. Gunsalus (*J. Biol. Chem.* **165,** *731* (*1946*)). You discuss the whole subject so well, and in particular the action of pyridoxal phosphate as a codecarboxylase and a co-transaminase, that the omission of this newly discovered action of pyridoxal phosphate seems to be an oversight.

Martin E. Hanke
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Note: (The role of pyridoxal phosphate in tryptophane formation was omitted inadvertently. In a review of the scope attempted, undoubtedly other omissions, either intentional or inadvertent, will be recognized. In the above case, however, it was not meant to minimize the importance of the work nor to detract from the excellent contributions made by the Cornell workers on the function of this coenzyme.—*Ed.*)

Proteins with Growth Promoting Activity

It is reasonable to believe that individual tissue cells are more fastidious in their growth requirements than the whole organism in which they occur, since individual tissues may depend for their growth and function on substances elaborated by other tissues of the body, in addition to nutrients obtained from the diet. It is only necessary to point out the significance of the glands of internal secretion for the normal function and even the survival of the rest of the organism. C. Waymouth (*Proc. Soc. Exp. Biol. Med.* **64,** *25* (*1947*)) has extended the studies of A. Fischer and T. Astrup (*Pflügers Arch. f.d. ges. Physiol.* **247,** *34* (*1943*)) on a protein component of embryo extract which promotes the growth of cells cultured *in vitro*. The activity is measured by observing the increase in area of pieces of explanted tissue. Although an increase in area does not necessarily signify an increase in mass, it does represent a biologic response which is nonetheless of interest.

Waymouth found that the most active preparations contained nucleoprotein chiefly of the ribonucleic acid type. Activity was not lost through dialysis or by removing lipids by precipitating with organic solvents at −15°C. Activity was, however, destroyed by heat, proteolytic enzymes, or by all attempts to dissociate the protein and nucleic acid. It was possible to obtain active material from saline extracts of either chick or calf embryo. The material from calf embryo had a sedimentation constant indicating that it had a molecular weight comparable to that of the albumins. Similar preparations from certain adult tissues also contained activity and the suggestion was made that the material or materials responsible may be widely distributed in adult tissues but are sometimes masked by the presence of large amounts of nonspecific proteins. The author is cautious in ascribing activity to a nucleoprotein until preparations of greater purity are obtained.

Caloric Intake and Longevity

C. M. McCay and co-workers (*Nutrition Reviews* **2,** *3* (*1944*)) showed in a series of investigations that the life span of rats receiving a reduced caloric intake was increased considerably over that of animals fed ad libitum. This work was done with a natural type ration. W. H. Riesen, E. J. Herbst, C. Walliker, and C. A. Elvehjem (*Am. J. Physiol.* **148,** *614* (*1947*)) report results of a similar study carried out with a purified ration containing: sucrose 48; casein 36; salts 8; corn oil 6; liver powder 2; with thiamine, riboflavin, pantothenic acid, niacin, pyridoxine, choline, and fat soluble vitamins added in adequate amounts.

Three groups of rats (20 animals in each)

were used simultaneously. One group, called the maintenance group, received the basal ration at a level just sufficient to allow slow growth. The total caloric intake of this group averaged 28 calories per day over the greater portion of their life span. A second group, the restricted group, received the same ration at an average level of 40 calories per day. The control group (unrestricted) received the same amount of the ration as the restricted group, but was allowed additional sucrose ad libitum; their intake averaged 52 calories per day during their life span. Growth of the animals increased with the caloric intake. The experiment was continued for one hundred weeks, and records of all fatalities kept. At the end of the experimental period, 4, 2, and 12 animals remained alive in the control, restricted, and maintenance groups, respectively. The control group showed the largest number of deaths early in the life span, the maintenance group least, and the restricted group an intermediate number. The incidence of tumors and of fatty degeneration of the organs was also lowest in the maintenance group, intermediate in the restricted group, and highest in the control group. The incidence of these conditions in all groups, however, was so low that definite conclusions should not be drawn.

The general conclusion of McCay and co-workers has therefore been confirmed, although perhaps less strikingly, a difference which may be dependent on the different types of diet.

Although the life span of the animals was increased by caloric restriction, the magnitude of the increase was less than that previously noted by McCay and co-workers. It would be interesting to know whether this difference is due to the different types of diets used.

The "Rice Diet" in Experimental Hypertension

During the past few years interest has been renewed in the dietary treatment of nephritis and hypertension, chiefly by the reports of successful treatment of patients with a diet of rice and fruit, which is low in protein and salt (W. Kempner, *N. Carolina Med. J.* **6,** *61 (1945)*). As in other instances, clinical progress may be hastened by more controlled studies in experimental animals. Experimental hypertension has been produced in animals by various technics which seem to have as a common basis the compression of the kidney (A. Grollman, *Proc. Soc. Exp. Biol. Med.* **57,** *102 (1944)*). A. Grollman and T. R. Harrison (*Ibid.* **60,** *52 (1946)*), employing rats made hypertensive by wrapping cotton thread around the kidney, demonstrated that a drastic reduction of sodium in the diet led to a marked drop in the blood pressure.

Another method of producing experimental hypertension, and possibly more related to the etiology of hypertension in man, was reported by G. F. Dick (*Arch. Path.* **39,** *81 (1945)*). This consisted of an intravenous injection of streptococci, which was found to result over a period of time in nephrosclerosis and rise in blood pressure. On such hypertensive dogs, G. F. Dick and W. B. Schwartz (*Proc. Soc. Exp. Biol. Med.* **65,** *22 (1947)*) have tested the effect of the so-called "rice diet."

Hypertension had been maintained in 12 dogs for from two to four years, with monthly recording of arterial pressure. During an eight week period on the "rice diet," one dog died and one showed only a negligible change in blood pressure, but the others showed an average diminution during the period from the initial 181.6 mm. to 138 mm.

The results appear significant, yet the experiments are only preliminary and without adequate control. No control dogs were used and no account is given of the amount of "rice diet" actually consumed. Actually the dogs lost an average of 19.7 per cent of their weight, and one dog refused to eat and died of starvation. It would be pertinent to know therefore what effect simple starvation would have on the blood pressure. The clinical implications of the study certainly demand a thorough investigation of the reported results.

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